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Seroprevalence of IgG antibodies against *Anaplasma marginale* in cattle from south Mozambique

Soroprevalência de anticorpos de classe IgG contra *Anaplasma marginale* em bovinos da região Sul de Moçambique

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Abstract

The current study aimed to investigate the seroprevalence of IgG antibodies to *Anaplasma marginale* in cattle from Maputo, Gaza and Inhambane provinces, south Mozambique. A total of 809 serum samples from cattle were obtained and tested by indirect enzyme-linked immunosorbent assay (i-ELISA). The chi-square test at 5% significance was used to assess the association between seroprevalence and the variables gender, age and geographic origin of animals. The overall seropositivity was 76.5% (n = 619) and anti-*A. marginale* antibodies were detected in 89.1% (n = 156), 68.4% (n = 308) and 84.2% (n = 155) of the animals in the provinces of Maputo, Gaza and Inhambane, respectively. A significant association (p < 0.05) was found with the geographic origin of the animals, while sex had no significant relationship. The frequencies of seropositive in the age groups were 63.2% (n = 72), 80.0% (n = 92), 83.1% (n = 98) and 77.3% (n = 357) for animals <12; >12 and ≤24; >24 and ≤36; >36 months, respectively. These results indicate that in southern Mozambique there are areas of enzootic stability to *A. marginale*. Thus, epidemiological monitoring is required to monitor the immune status of animals in the region.

Keywords: Bovine anaplasmosis, epidemiology, serological, Mozambique.

Resumo

O objetivo do presente estudo foi investigar a soroprevalência de anticorpos da classe IgG contra *Anaplasma marginale* em bovinos de corte da região Sul de Moçambique. Para esse efeito, 809 amostras de soro foram coletadas e avaliadas pelo ensaio imunoadsorção enzimático indireto (ELISA-i). O teste Qui-Quadrado, a 5% de significância, foi utilizado para avaliar a associação entre a soroprevalência e as variáveis sexo, idade e origem geográfica dos animais. A soropositividade geral foi de 76,5% (n = 619), e anticorpos anti-*A. marginale* foram detectados em 89,1% (n = 156), 68,4% (n = 308) e 84,2% (n = 155) dos animais nas províncias de Maputo, Gaza e Inhambane, respectivamente. Uma associação significativa (p < 0,05) foi observada entre a origem geográfica dos animais, enquanto o sexo não demonstrou uma relação significativa. A frequência de soropositivos com relação à faixa etária foi de 63,2% (n = 72), 80,0% (n = 92), 83,1% (n = 98) e 77,3% (n = 357) para animais de <12; >12 a ≤24; >24 ≤36; >36 meses, respectivamente. Os resultados demonstram que, no Sul de Moçambique, existem áreas de estabilidade enzoótica para *A. marginale*, em animais maiores de 12 meses. Assim, monitoramento epidemiológico deve ser realizado para o acompanhamento do status imunológico dos animais na região.

Palavras-chave: Anaplasmoze bovina, epidemiologia, sorologia, Moçambique.

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Introduction

Livestock production has grown significantly since the mid-1990s in Mozambique as a result of the importation of cattle from neighboring countries in the implementation of livestock restocking program. However, this increase in cattle production was not followed by improvement of sanitary conditions of breeding animals, which has led to the occurrence of many diseases, among which are noteworthy the ones transmitted by ticks, such as anaplasmosis, babesiosis, ehrlichiosis and theileriasis (SIMUUNZA et al., 2011). These diseases have led to a decrease in productive and reproductive efficiency of livestock, in addition to mortality of more than half of the animals (MARTINS et al., 2008).

Anaplasmosis causes severe damage to animal health and has great economic impact on livestock in tropical regions (ALFREDO et al., 2005; JONSSON et al., 2008). Tick-borne diseases have been suspected as being responsible for 50% mortality in cattle (MARTINS et al., 2008). The dynamics of infection by *Anaplasma marginale* depends on several factors, including transmission capacity of the vector, susceptibility of cattle which can vary among race, age, and physiological and immune status. In addition, agro-ecological and edaphic-climatic conditions of each geographic region should also be considered (BOCK et al., 1997; JONSSON et al., 2008). Anaplasmosis is transmitted by ixodid ticks of the genus *Rhipicephalus*, *Hyalomma* spp., *Dermacentor* spp., especially *Rhipicephalus microplus* (UILENBERG, 1995; DE WALL, 2000; JONGEJAN; UILENBERG, 2004). Other forms of transmission include mechanical means by hematophagous arthropods such as *Tabanus*, *Stomoxys*, and mosquitoes (SCOLES et al., 2005), fomites (REEVES; SWIFT, 1977) and through placenta during pregnancy (POTGIETER; VAN RENSBURG, 1987). In Mozambique, *A. marginale* transmission vectors are ticks of the genera *Rhipicephalus*, *Hyalomma* spp. and other arthropods, such as a horsefly *Stomoxys*, and mosquitoes. Fomites, mainly due to mandatory vaccination in the country, are also important (SCOLES et al., 2005). In addition, direct losses due to mortality caused by anaplasmosis are estimated to be more than 50% of domestic bovine animals (MARTINS et al., 2008).

This pathogen causes massive extravascular destruction of red cells in their hosts; autoantibodies appear with disease progression and adhere to infected and uninfected erythrocytes, increasing red blood cell phagocytosis by macrophages, mainly in the spleen and bone marrow. Reduced corpuscular volume and progressive anemia occur and may lead to death (RISTIC, 1960).

The seroepidemiological study of bovine anaplasmosis in a given geographical area is important because it can demonstrate the possibility of outbreak occurrence. Indirect methods, which detect antibodies against *A. marginale* surface antigens, are essential tools for assessing the prevalence and the immune status of animals and can provide useful information for the control of tick vectors (MAHONEY, 1975; POTGIETER, 1979). Most common serological tests include rapid card agglutination test, indirect immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA). Moreover, several researchers have demonstrated that indirect ELISA is the test with higher diagnostic sensitivity

and specificity (KNOWLES et al., 1996; GITAU et al., 1997; TRUEBLOOD et al., 1991; MADRUGA et al., 2000).

The current study aimed to investigate the prevalence of IgG antibodies against *A. marginale* by indirect ELISA, and to assess whether gender, age and geographic area of origin are potential risk factors in cattle from the provinces of Maputo, Gaza and Inhambane, south Mozambique.

Materials and Methods

1. Study area

The study was conducted in the provinces of Maputo, Gaza and Inhambane located in the southern region of Mozambique (25° 58" S latitude, 32° 35' E longitude, 120 m altitude), with an area of approximately 167,641 km² and a population density of 29.1 inhabitants/km² (INE, 2010). The area comprises 53.6% of national cattle population, estimated in 902,579 heads (INE, 2008). The districts studied in each province were: Magude, Moamba and Namaacha in Maputo; Bilene, Chibuto, Chokwé and Xai-Xai in Gaza; and district of Zavala in Inhambane (Figure 1).

The climate is predominantly tropical and humid, with warm summer and cold winter. The average temperature ranges from 24 to 40 °C during the summer (October to April) and 18 to 23 °C during the winter (May to September). In addition, average precipitation ranges from 600 to 800 mm and occurs mostly between October and April.

2. Animals and serum samples

Serum samples were obtained from a total of 809 *Bos indicus* cattle from 110 rural smallholder communities located in the provinces of Maputo, Gaza and Inhambane, south Mozambique. Animals were raised in plain agro-ecological areas under different management systems. However, extensive management system predominated. The study was carried out between January and March 2010 and cattle owners participated voluntarily in this study.

Non-probability convenience sampling was used following proportional stratification for each administrative region studied. In each rural smallholder community samples were taken in order to represent 10% or more of the total herd in each farm. The number of blood samples was calculated using an expected prevalence of 34% (ALFREDO et al., 2005), sampling error of 3.5%, according to the equation described by Sampaio (2002): $n = z^2 \cdot p(1-p)/d^2$, where n = sample size; z = constant 1.96; p = expected prevalence, d^2 = desired absolute precision.

Blood samples were collected aseptically by venipuncture of the coccygeal vein into 10 mL tubes without anticoagulant using vacuum system. Serum samples were obtained by centrifugation at 3000 rpm for 10 minutes and were stored at -20 °C until serological assays were performed.

The animals were categorized by age (<12 months; >12 and ≤24 months; >24 and ≤6 months and >36 months), sex (males and females) and geographic origin (Maputo, Gaza and Inhambane).



Figure 1. a) Map of Africa and geographical location of Mozambique; b) Map of Mozambique indicating the southern region; c) Study sites in three provinces. 1. Chitsuleta, 2. Nhamaso-Chambule, 3. Chissubuca, 4. Bilene, 5. Chokwé, 6. Chibuto, 7. Xai-Xai, 8. Magude, 9. Moamba, 10. Namaacha.

3. Enzyme-linked immunosorbent assay (ELISA)

Indirect ELISA was performed to detect anti-*A. marginale* IgG antibodies according to Araújo et al. (2005). Optimization of the reaction was established in preliminary experiments through block titration of reagents, testing of different antigen concentrations, positive and negative control serum dilutions and enzymatic conjugate dilutions. Microplates (Corning® Costar® 3590, USA) were coated with recombinant MSP5 of *A. marginale*, supplied by the Brazilian Agricultural Research Corporation (Embrapa) Beef Cattle, Mato Grosso Sul, Brazil, and then blocked with PBS Tween-20 at 0.1% (PBS-T) and skim milk at 5% (PBS-TM). Next, plates were incubated with cattle sera diluted at 1:800 in PBS-T, followed by incubation with anti-bovine IgG horseradish peroxidase conjugate (Sigma Chemical Co, USA), diluted at 1:10,000 in

PBS-T. The reaction was developed by adding the enzyme substrate (o-phenylene diamine dihydrochloride (OPD; Sigma) and was stopped by the addition of H_2SO_4 (2.5 N). The optical density (OD) was read at 492 nm in plate reader (Lab systems IEMS Reader MF). Positive and negative control sera were included in each plate and all tests were performed in duplicates. The reaction cutoff was set as two and a half times the mean absorbance of the negative group control sera, and all readings above the cutoff were considered positive. The immunological reactivity of each serum was calculated by determining the sample to positive serum ratio (S/P), considering positive and negative sera as reference, using the following equation: $(\text{mean sample absorbance} - \text{mean absorbance of negative serum reference}) / (\text{mean absorbance of positive reference serum} - \text{mean absorbance of negative serum reference})$, as described by Machado et al. (1997).

4. Statistical analysis

The frequencies of seropositivity according to different categories (age, gender and origin) were calculated and evaluated by the chi-square test (χ^2) at a significance level of 5% using the statistic program BioEstat version 4.0 (AYRES et al., 2005).

Results

Of the 809 sera analyzed by ELISA, 76.5% (n = 619) showed anti-*A. marginale* IgG antibodies. The seroprevalence of bovine anaplasmosis in the provinces of Maputo, Gaza and Inhambane and the association with different factors studied are shown in Table 1.

Regarding cattle age, positive sera were found in all age groups and anti-*A. marginale* seropositivity was associated ($p < 0.05$) with age. Cattle aged up to 12 months had lower seropositivity compared to those aged between 12 and 24 months, 24 and 36 months and animals older than 36 months, being 63.2% (n = 72), 80.0% (n = 92), 83.1% (n = 98) and 77.3% (n = 357) positive for anti-*A. marginale* IgG antibodies, respectively. The prevalence ratio (Table 2) showed that cattle aged over 12 months were 4 to 27% more likely to be exposed to *A. marginale* ($p < 0.01$). As for animal gender, no association ($p > 0.05$) with seropositivity to *A. marginale* was found.

Maputo province had a higher frequency rate ($p < 0.05$) of seropositive cattle by ELISA compared to the two other provinces and anti-*A. marginale* antibodies were detected in 89.1% (n = 156), 84.2% (n = 155) and 68.4% (n = 308) of the animals in Maputo, Inhambane and Gaza, respectively (Table 1). The prevalence rate showed that cattle in the provinces of Maputo and Inhambane were 6 and 23% ($p < 0.01$) more likely to be exposed to *A. marginale* (Table 2).

The frequency of bovines positive to *A. marginale* in the districts followed the same pattern observed for the provinces (Table 1). However, the Moamba district in the province of Maputo had the highest frequency of seropositive cattle, 93.8% (n = 45). On the other hand, Chibuto district in the province of Gaza, had the lowest frequency of seropositive cattle, 47.9% (n = 78).

Discussion

In the current study the occurrence of antibodies against *A. marginale* in cattle from south Mozambique was investigated by ELISA. The study results demonstrated that these ruminants are exposed at a high frequency to this hemoparasite in the region studied. It is the first evidence of this agent in the region.

Anti-*A. marginale* IgG antibodies were detected in 76.5% (n = 619) of the animals, of which 89.1% (n = 156), 68.4% (n = 308) and 84.2% (n = 155) were in the provinces of Maputo, Gaza and Inhambane, respectively. This seropositive rate is higher than 34.4% reported by Alfredo et al. (2005) in a similar study conducted in the province of Tete, central Mozambique, in areas of semi-arid and arid climate and high altitude. Moreover, the prevalence of *A. marginale* in the current study was higher than 75.0% in all districts except Chibuto, located in Gaza province

Table 1. Prevalence of antibodies against *Anaplasma marginale* in cattle from Maputo, Gaza and Inhambane provinces, southern Mozambique, detected by indirect enzyme-linked immunosorbent assay (i-ELISA) and factors associated.

Factor	N	<i>Anaplasma marginale</i>	
		n	%
Prevalence	809	619	76.5
Age (months)			
<12	114	72	63.2 ^a
>12 to ≤ 24	115	92	80.0 ^b
>24 to ≤ 36	118	98	83.1 ^b
>36	462	357	77.3 ^b
Gender			
Female	495	379	76.6 ^a
Male	314	240	76.4 ^a
Maputo	175	156	89.1 ^A
Moamba	48	45	93.8 ^a
Magude	79	70	88.6 ^b
Namaacha	48	41	85.4 ^b
Inhambane	184	155	84.2 ^B
Chitsuleta	61	50	82.0 ^a
Nhamaso-Chambule	59	51	86.4 ^a
Chissibuca	64	54	84.4 ^a
Gaza	450	308	68.4 ^C
Bilene	104	92	88.5 ^a
Chokwé	47	36	76.6 ^a
Xai-Xai	136	102	75.0 ^a
Chibuto	163	78	47.9 ^b

N = number of animals by factor; n = number of samples positive to *A. marginale*. The values in the same column followed by a different letter show a statistically significant difference among groups or factors in test χ^2 ($p < 0.05$).

(Table 1), which showed a rate of 47.9%. This lower prevalence rate is probably due to the use of intensive health management in Chibuto, characterized by acaricide dip at weekly intervals, possibly affecting the population of vector ticks.

In southern Mozambique cattle raising is predominantly beef cattle, consisting mostly of *Bos indicus* breeds and their crosses, which are naturally tolerant to tick parasitism (JONSSON et al., 2008). Hence, the results of the current study demonstrate great transmission potential in the area studied and points out the importance of control measures.

Studies in other countries in the Eastern and Southern regions of Africa indicate that *A. marginale* is widely distributed with a prevalence ranging from 32.1 to 100.0% (LATIF et al., 1995; DREYER et al., 1998). These results demonstrate not only areas of stability, but also situations of enzootic instability between countries or regions, which may be associated to hygienic-sanitary management and soil and edaphic-climatic conditions that affect the survival of *A. marginale* vectors (SCOLES et al., 2005; JONSSON et al., 2008). In Zambia, the prevalence of *A. marginale* demonstrated by ELISA was 85.7, 85.9, 84.7 and 75.0% in provinces located at Western, Central, Eastern and Southern areas of the country, respectively (JONGEJAN et al., 1988). In addition, several studies in South Africa using ELISA as the diagnostic test reported

Table 2. Prevalence ratio of antibodies against *Anaplasma marginale* in cattle from Maputo, Gaza and Inhambane provinces, southern of Mozambique, detected by indirect enzyme-linked immunosorbent assay (i-ELISA) and factors associated.

Factor	N	<i>Anaplasma marginale</i>				
		n ^a	% ^b	PR	95% CI ^c	^d p-value
Age (months)						
<12	114	72	63.2	1		
>12 to ≤24	115	92	80.0	1.27	1.07-1.50	0.00
>24 to ≤36	118	98	83.1	1.04	0.92-1.17	0.33
>36	462	357	77.3	1.07	0.98-1.18	0.11
Province						
Gaza	450	308	68.4	1		
Inhambane	184	155	84.2	1.23	1.13-1.34	0.00
Maputo	175	156	89.1	1.06	0.98-1.15	0.11
Districts						
Moamba	48	45	93.8	1		
Magude	79	70	88.6	1.06	0.95-1.18	0.26
Namaacha	48	41	85.4	1.10	0.96-1.26	0.16
Bilene	104	92	88.5	1.06	0.96-1.17	0.23
Nhamaso-Chambule	59	51	86.4	1.02	0.91-1.16	0.45
Chissibuca	64	54	84.4	1.02	0.89-1.19	0.47
Chitsuleta	61	50	82.0	1.03	0.88-1.21	0.45
Chokwé	47	36	76.6	1.22	1.03-1.46	0.02
Xai-Xai	136	102	75.0	1.25	1.11-1.41	0.01
Chibuto	163	78	47.9	1.96	1.64-2.34	0.00

N = Number of animals by factor; PR = Prevalence ratio; ^aNumber of positive samples; ^bPrevalence; ^cConfidence interval and ^dp-value.

seropositive rates for bovine anaplasmosis ranging from 87 to 100% (DREYER et al., 1998; MBATI et al., 2002; MTSHALI et al., 2004; MTSHALI et al., 2007; NDOU et al., 2010).

In the United Republic of Tanzania, the prevalence of *A. marginale* was reported as being 37.0 and 40.0% in Iringa and Tanga region, respectively (SWAI et al., 2005). Moreover, in Kenya, in the East Africa region, a seroprevalence of 32.1 to 36.0% was observed, which is much lower than that reported in other African areas (LATIF et al., 1995; OKUTHE; BUYU, 2006). Therefore, differences in prevalence of bovine anaplasmosis between different geographic regions of the same continent may occur.

In the current study, no association ($p > 0.05$) was seen between seropositivity to *A. marginale* and gender. These results demonstrate that infection with *A. marginale* in the provinces of Maputo, Gaza and Inhambane is independent of this parameter (Table 1), a finding that is consistent with other studies (SOARES et al., 2000; SOUZA et al., 2000; TRINDADE et al., 2010). On the other hand, statistical significant difference in the prevalence of *A. marginale* was found among the three age groups studied (Table 1). Similar results were reported by Bock et al. (1997) who showed that factors such as race, age, physiological and immunological status may affect seropositive rates. It is noteworthy that parasite inoculation rate by other biological vectors (DREYER et al., 1998; MTSHALI et al., 2007; NDOU et al., 2010) and other sources of mechanical transmission via blood-sucking dipterans and fomites (SCOLES et al., 2005) may also affect the enzootic stability of any geographic area when *A. marginale* is prevalent. Additionally, the lowest seropositivity ($p < 0.05$) seen in cattle aged up to 12 months can be explained by immune protection due to colostral antibodies and/or low rate of inoculation of *A. marginale* by the

vectors as the animals in this age group have much lower contact with the vectors compared to older ones (JONSSON et al., 2008).

There were only a few previous reports on the prevalence of bovine anaplasmosis in Mozambique in other areas (ALFREDO et al., 2005). To our best knowledge, this is the first report on the prevalence of *A. marginale* in Maputo, Gaza and Inhambane provinces, Mozambique. These data may provide valuable input to managers of national livestock and can help understanding the immunological status of herds as well as planning future interventions for animal health. Further studies are needed to monitor herds and identify other factors that may pose an epidemiological risk in the region.

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